CHANGES IN THE TISSUE CHLORIDE OF THE CALIFORNIA MUSSEL IN RESPONSE TO HETEROSMOTIC ENVIRONMENTS [1]

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The adult California mussel, *Mytilus californianus* Conrad is a fairly heterosmotic animal, typically marine in its environment, yet potentially euryhaline in a striking degree.

It has been shown (Fox et al., 1936) that this mollusk can live in the laboratory for long periods following sudden and continued immersion in aerated solutions of natural sea salts, varying in Cl concentration [2] from about 0.94 per cent, or approximately half that of natural sea water in the vicinity of La Jolla, to as high as 2.5 per cent, or about 34 per cent above normal values (1.86 per cent being the approximate Cl concentration of sea water at La Jolla).

Sudden exposure to concentrations of sea salts below or above these respective values proved fatal to mussels, but gradual alteration of the water in which they were immersed, over periods of several weeks, left the animals still alive in solutions diluted, on the one hand, to one-third of the normal Cl concentration (0.62 per cent Cl) or concentrated, on the other hand, to nearly twice the normal value (3.50 per cent).

The purpose of this investigation was to determine the chloride concentration in the tissues of the mussel, under the widely differing physiological conditions which must result from continued exposure to concentrations of sea salts not encountered by these animals in nature.

Extensive reviews of both older and more recent work dealing with homoiosmoticity and heterosmoticity of fishes and invertebrates have been presented in papers by Dakin (1935) and by Schlieper (1935).

[1] Contributions from the Scripps Institution of Oceanography New Series No. 117.

[2] Cl concentration refers to the total halide ion concentration as determined in sea water analyses. The same designation, used in the present discussion, refers to the total halide concentration in parts per cent of both water and tissues, since the amounts of both Br and I are relatively very small. Concentration of "chloride" refers hereinafter to that of halide ions.

Other useful discussions of the subject are given by Adolph (1930) and by Baldwin (1937).

Experimental Methods

Diluted sea water solutions were prepared by adding distilled water to sea water, while relatively concentrated solutions were obtained by the partial evaporation of ordinary sea water without precipitating any salts.

Experimental solutions were contained in glass battery jars, each of 2½ gallon capacity. To avoid any injurious effects which might result from overcrowding, the predetermined ratio of at least one liter of water per animal was consistently adopted as a minimum. Mussels of an average length of 10 cm. (varying between 9 and 11 cm.) were employed in the great majority of experiments, although no biochemical or physiological differences of direct bearing upon the work were recognized in larger animals (12 cm.) or in somewhat smaller ones (7 to 8 cm.)

Experiments were carried out at room temperatures which varied between the approximate limits of 19° and 22° C. All solutions containing mussels were aerated continuously.

No attempts were made to adjust and maintain the pH of the various solutions, since it was found that mussels lived in piped sea water within the range of pH values encountered in the experiments. Figures representing numerous pH measurements of environmental solutions will serve as examples and are tabulated below. These measurements were made with a Beckman glass-electrode pH meter through the kindness of Mr. J. C. Hindman.

Solution	pH
(1) Sea water of normal salinity from running supply in tank containing mussels; unaerated	8.63
(2) The same; aerated	8.43
(3) The same, drained from mantle-cavity of mussel; unaerated	7.51
(4) Sea water of normal salinity from stationary supply in large jar containing mussels; unaerated	7.50
(5) Sea water, initially of normal salinity, diluted to maximum degree used in experiments, i.e. to one-third of original concentration (ca 0.62 per cent Cl); aerated	8.52
(6) Sea water, initially of normal salinity, concentrated to maximum degree used in experiments, i.e. to 54 per cent of original volume (ca 3.50 per cent Cl); aerated	8.32

Since the mussels were therefore presumed not to have been exposed to unfavorable conditions of acidity or alkalinity during the course of the experiments, it was concluded that extensive changes in the concentration of dissolved salts themselves constituted the limiting factors to life in the environment. The possibility of disturbances in " salt balance " (in the physiological sense), of experimental solutions was considered. Because calcium was believed to be the chief element prone to be precipitated as the carbonate from moderately concentrated sea water, analyses were made for dissolved calcium in sea water samples concentrated by boiling. Normally present in amounts close to 0.42 gram per liter, the dissolved calcium in a solution boiled down without precipitation to 54 per cent of its original volume (number 6 in above table) was present in the nearly theoretical amount of 0.80 gram per liter. Since this solution was the most concentrated used in any of the experiments, it was concluded that mussels were at no time exposed to solutions " unbalanced " with reference to calcium.

Experimental animals were introduced into the various solutions of altered salinity after first propping the valves apart by a few millimeters with smooth glass plugs and draining the gill chamber of sea water. Animals immersed under these conditions in sea-salt solutions varying between the approximate limits of 0.95 per cent and 2.73 per cent Cl usually relaxed their hold on the glass rod in a short time, parted their valves in a normal manner, and resumed feeding activities.

The flesh of control and experimental mussels was prepared for chloride analysis in the following way. The mussels were removed from their shells as rapidly as possible and with minimum cutting of tissues, severing only the adductors and small muscles attached to the hinge region of the shells. The flesh was blotted on absorbent paper to remove most of the adhering sea water, then rinsed briefly in 95 per cent ethyl alcohol (and reblotted) in order to remove most of the remaining sea water from body surfaces, constrict the gill capillaries, wash out sea water expressed therefrom, and coagulate the cut surfaces to allay excessive bleeding and subsequent losses of chloride-containing body fluids. The consistent adoption of this procedure resulted in a series of checks which were quite close in normal control animals, in spite of the fact that the work was done on the wet weight basis.

For chloride analysis, the method of Sunderman and Williams (1931, 1933) was followed, digesting the whole tissues in chloride-free KOH, followed by further treatment of aliquot portions with concentrated HNO_3 in the presence of an excess of dilute $AgNO_3$ solution. The excess of Ag ion was finally titrated with standard NaCNS solution in

TABLE I

Chloride concentrations in wet tissues of mussels, showing slight variations with sex, maturity and season.

Date	No.	Sex	Weight of flesh	Percentage Cl		
			grams			
8–11–37	1	♂ (much sperm)	22.88	0.99		
8–13–37	2	♂ " "	18.70	0.83		
8–13–37	3	♀ (many eggs)	14.85	0.89		
"	4	♂ (much sperm)	16.57	0.89		
"	5	♀ (many eggs)	16.50	0.82		
"	6	♀ " "	13.23	0.805	av. % Cl in whole ripe ♂ = 0.91	
"	7	♂ (much sperm)	17.59	0.83	av. % Cl in whole ripe ♀ = 0.87	
8–17–37	8	♂ (mature)	12.51	1.00	Grand av. = 0.90	
"	9	♂ "	10.025	0.965		
"	10	♀ "	8.52	1.065		
"	11	♂ "	11.50	0.81		Summer.
"	12	♀ "	10.61	0.77		
"	13	♂ (immature)	6.56	0.99		
	Gonads only	♂	16.95	0.90		
	" "	♀	15.68	0.61		
	" "	♂	15.03	0.945	av. % Cl in ripe ♂ gonad = 0.89	
	" "	♀	13.86	0.58	av. % Cl in ripe ♀ gonad = 0.57	
	" "	♂	10.24	0.82		
	" "	♀	13.61	0.52		
12–13–37	1	? (immature)	6.41	0.97		
"	2	♂ "	6.26	0.98		
"	3	? "	7.98	0.96		
"	4	? "	8.19	0.98	av. % Cl in immature animals = 0.98	
"	5	♂ "	5.96	1.005		
"	6	♂ "	5.08	0.995		
1–31–38	1	♀ (many eggs)	12.41	1.00		
"	2	♀ (spawned)	9.84	0.91		Winter.
"	3	♂ (much sperm)	16.37	0.96		
"	4	♂ " "	18.16	0.97	av. % Cl of sperm—cont'g ♂ = 0.97	
"	5	♀ (many eggs)	12.08	0.93	av. % Cl of egg—cont'g ♀ = 0.93	
"	6	♂ (much sperm)	15.69	1.01	av. % Cl of all ♀ = 0.945	
"	7	♀ (many eggs)	17.94	0.905	av. % Cl of all animals = 0.96	
"	8	♂ (much sperm)	16.49	0.945		
"	9	♀ (many eggs)	21.20	0.90		
"	10	♀ (spawned)	12.38	1.03		

the presence of ferric alum according to the well-known method of Volhard. The Volhard method was also employed for the analysis of chloride in sea-water solutions. Our experiments showed that the step involving preliminary digestion of tissues by KOH gave consistently higher values for chloride than did the ordinary "open Carius" determinations (digestion with excess concentrated HNO_3 and $AgNO_3$) employed by other workers. Sunderman and Williams (1933) report incomplete recovery of chloride when the preliminary alkaline digestion is omitted, and assign the low chloride values to interference by fatty substances.

Preliminary Analytical Survey of Normal Animals

Because parts of this research were conducted in different seasons of the year, i.e. especially in the summer and fall of 1937 and the winter of 1937–1938, it seemed desirable to compare chloride analyses of normal animals taken during August with those of animals comparable in size and weight taken in December and January. Also, because it was impossible to differentiate the sexes without sacrificing the animals' lives, and since it was conceivable that biochemical differences in sex might be reflected in the chloride content of the tissues, attention was given to the sex and relative degree of maturity throughout the same group of animals.

The data of Table I reveal that differences between the chloride content of whole bodies of summer animals and those of winter animals are of relatively small order, showing a departure of only + 0.06 per cent in the grand average, in favor of the winter animals; this difference is the same in direction and extent whether one compares ripe winter with ripe summer males, or ripe winter with ripe summer females.

Sexually immature animals exceeded in chloride content the grand average (0.93 per cent) of the combined values of mature animals of both seasons by the small departure of 0.05 per cent.

Because the demonstrated seasonal and sexual differences in chloride content fell well within the departures recorded between individual analyses, they were not regarded as significant in the experimental results. The sexual difference in chloride content appeared to be a real one, although relatively small, and for the purposes of this work, insignificant. It was doubtless due to the fact that the relatively heavy ripe ovary contained only about two-thirds as much chloride as did the male gonad.

Changes in Tissue Chloride Following Sudden Immersion in Heterosmotic Solutions of Sea Salts within the Tolerated Physiological Range of Concentrations

Reference to Figs. 1 and 2 brings out some rather consistent general facts: While mussels in nature show close agreement among one another

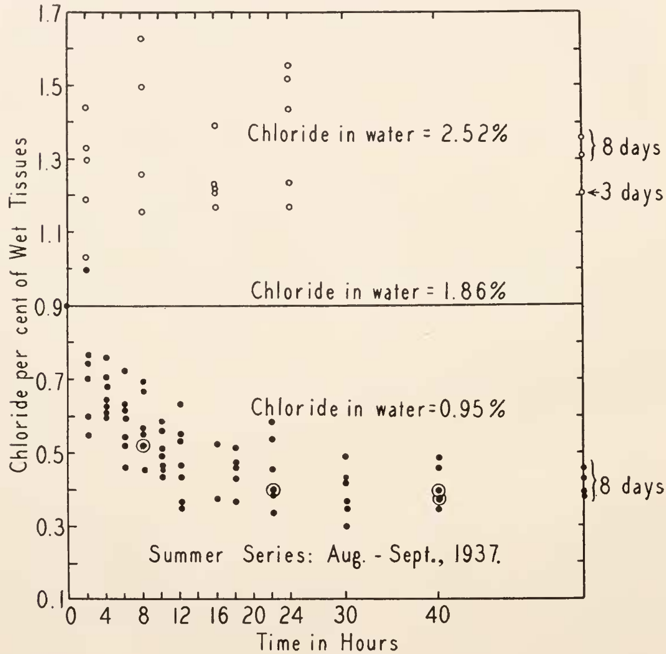

FIG. 1. Chloride analyses of whole mussel tissues following immersion of living animals for increasing time-intervals in hypotonic and hypertonic sea water within the limits of the tolerated range. Each point represents the analysis of a single animal; where two or more analytical values were identical, this is shown by concentric circles.

in chloride content, wide individual variations are manifest in the rate of change in tissue chloride concentrations in both kinds of new environment. This is particularly striking in the early hours following immersion. Since mussels have been demonstrated to survive for long periods at either the hypotonic or the hypertonic concentrations here employed, and to eventually attain, in each group, respective chloride levels in close individual agreement, the obvious differences in the early hours are clearly due to individual variations in rate of water and salt interchange.

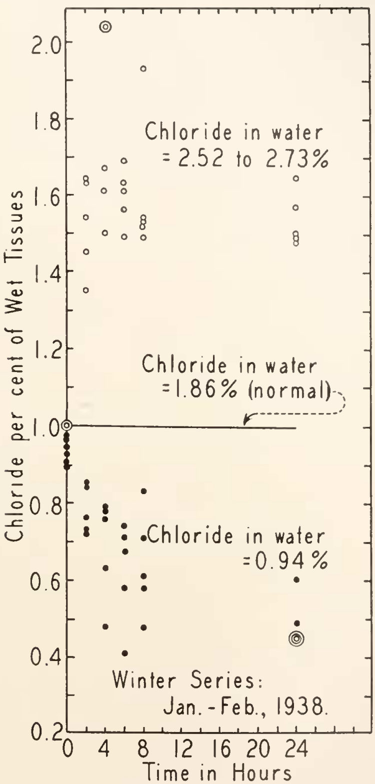

FIG. 2. Further chloride analyses, as in Fig. 1.
See Fig. 1 for meaning of points and concentric circles.

While four out of five animals which remained for the whole 24-hour period in water containing 2.52 to 2.73 per cent Cl (Fig. 2) were filtering water, feeding, and voiding feces and thus appeared normal, none of the animals in the water containing 2.78 per cent Cl (Fig. 3) appeared normal even after 30 hours; they maintained a grip on the inserted glass props, failed to filter water, and gave off much mucus, thus giving several signs of physiological disturbance. A later lot, however, exposed to a solution of 2.80 per cent Cl (Table IV) did not show any effects of injury after 25 hours. These facts are taken as evidence that Cl values between 2.70 per cent and 2.80 per cent are close to the threshold of hypertonicity at which the mussels can withstand sudden immersion.

Figure 4, to which reference will be made below, reveals the close fit to a straight line *between water chlorinities tolerated by mussels after sudden immersion.* Attention is directed, for the moment, only to the

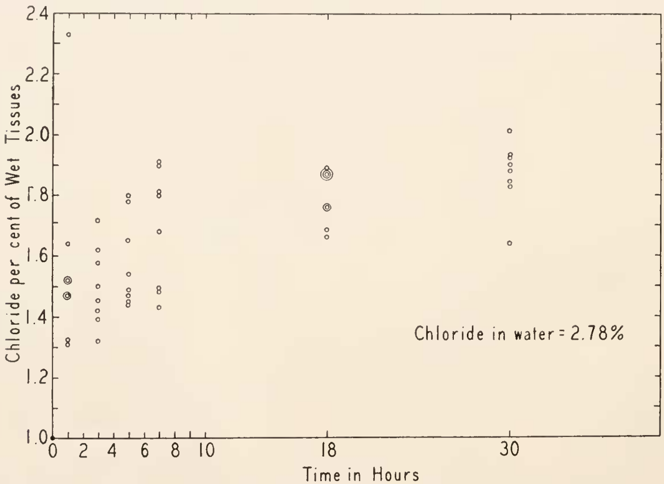

FIG. 3. Further chloride analyses, as in Figs. 1 and 2. See Fig. 1 for meaning of points and concentric circles.

portion of the graph lying between the ordinate values of 0.95 per cent Cl and 2.8 per cent Cl, illustrating the data shown in Figs. 1 and 2, and those of Table IV, when all average values of internal chloride concentrations attained by mussels in 24 to 40 hours are plotted against chloride concentrations of external water. The ratio between grams Cl per 100 grams wet mussel tissue and grams Cl per 100 ml. sea salt solution has an average value of 1 : 1.89.

The blackened point on Fig. 4 indicating the average chloride concentration in the tissues of 8 mussels maintained for 30 hours in water containing 2.78 per cent Cl was obtained from Fig. 3. While this average value lies in the vicinity of the curve, its departure is doubtless due to the fact that the animals were in poor condition (from an unknown cause), and so failed to maintain the ratio between internal and external chloride exhibited by other lots of animals at this concentration.

Specimens placed, with valves propped apart, in distilled water for 15- or 16-hour periods underwent a drop in tissue chloride to values of 0.226 per cent or even 0.112 per cent. Under these conditions animals, although not quite dead, were definitely moribund, and failed to recover when placed in running sea water.

Tolerance by the Mussel of Gradually Altered Concentrations of Sea Salts

While the immediately preceding experiments and earlier investigations seemed to define fairly well the limits of hypo- and hypertonic solutions withstood by mussels following *sudden* immersion, there were grounds for believing that *gradual* changes in the salt concentration in the animals' environment might not bring about signs of injury until greater extremes in both directions were reached.

In order to investigate this question, and to determine if possible the extremes of tissue chloride lost or gained by surviving animals, the following experiments were conducted. Two sets of mussels in separate jars of normal sea water, continuously aerated, were subjected to a gradual change in the chloride concentration of the water, one jar being diluted, the other concentrated daily by slow steps over a period of about six weeks. This gradual change was brought about in the following manner. From one jar containing about eight liters of sea water (seven mussels) some 250 ml. were withdrawn, discarded and replaced by an equal quantity of distilled water daily; from the other jar (eight mussels) the same volume of water was discarded and replaced by an equal quantity of water containing sea salts concentrated by about four-fold (i.e. Cl concentration of 7.725 per cent). These changes brought about a gradual drop in Cl from 1.86 to 0.625 per cent in the first, and a gradual rise from the same initial value to 3.48 per cent in the second solution, over the experimental period. Under these special conditions the mussels survived exposure to previously unexpected concentrations at both extremes.

Table II shows the results of chloride analyses, and a restoration of experimental animals to normal chloride levels after exposure to the gradually altered solutions during five and six-week periods.

It is assumed from the foregoing experiments that it has been possible to ascertain the approximate thresholds of tolerance of the mussel toward the osmotic effects of both hypotonic and hypertonic solutions of

TABLE II

Limits of tolerated changes in tissue chloride level.

Date 1937	Approximate time elapsed	Cl in hypotonic water	Cl in mussels	Cl in hypertonic water	Cl in mussels
		per cent		*per cent*	
Dec. 13, 14	5 weeks	0.84	3 animals: 0.41% 0.40 0.33 Average: 0.38%	3.09	5 animals: 2.08% 1.82 1.77 1.79 1.72 Average: 1.84%
Dec. 15	5 weeks	0.84	1 animal from same group, placed in running sea water 6 days, and analyzed: Cl = 1.1%	3.09	1 animal from same group, placed in running sea water 6 days, and analyzed: Cl = 0.935%
Dec. 20	6 weeks	0.625	0.26% (sluggish)	—	—
Dec. 21	6 weeks	0.625	0.53% (moribund) Remaining animal in this group placed in running sea water 13 days, and analyzed: Cl = 0.97%	—	—
Dec. 22	6 weeks	—	—	3.48	2 remaining animals (becoming sluggish) 2.19% 2.32 Average: 2.25%

sea salts. It seemed hardly likely, however, that individuals kept in solutions of concentrations below 0.94 per cent Cl or above 2.8 per cent Cl would survive indefinitely, since the flesh of animals in solutions beyond these respective concentrations appeared rather thin, sometimes even emaciated. Furthermore, they failed, in these respective realms of salt concentrations to maintain their constant ratio of tissue chloride to sea water chloride as illustrated in Figure 4. This graph summarizes much of the information reported above and indicates the relationship which exists between the chloride concentration in the surrounding media and that in the tissues of animals immersed therein. Each point

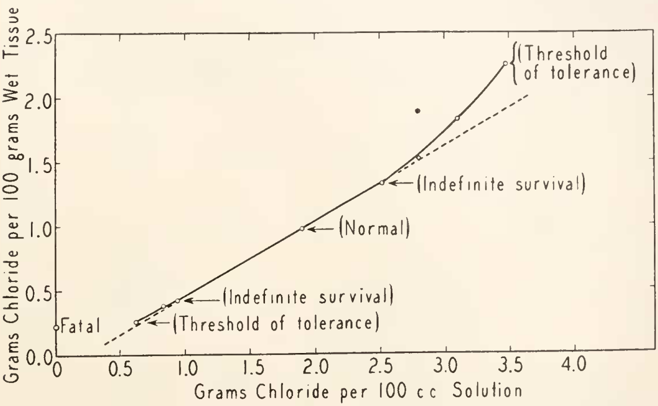

FIG. 4. Relationship between internal and external chloride, in grams Cl per 100 grams tissue vs. grams Cl per 100 ml. water respectively. Points lying between abscissal values of 0.95 and 2.8 represent results obtained after 24 hours or more following *sudden* immersion. Portions of the graph lying below and above these respective values represent the trend taken following gradual alteration of Cl concentration in the water over protracted periods, excepting wherein *distilled* water was used (see text).

represents the average of the analyses of a number of animals immersed in the respective solutions for 24 hours or more, save in the cases of the two lowest points on the hypotonic side, which represent analyses of two respective animals: a single survivor containing 0.26 per cent Cl and the animal which showed the highest Cl value (0.226 per cent) of the group that succumbed to immersion in distilled water (at zero abscissal value). The maintenance of a ratio of 1 : 1.89 between internal and external chloride is noted in the straight line between the latter concentrations of 0.94 per cent and 2.8 per cent Cl. The projection of this straight line as a dotted line beyond these values shows, when compared with the actual curve, the degree of departure in the animals' maintenance of such a ratio. The final two points at each extremity of the curve represent Cl values attained by mussels after *gradual* changes in Cl concentration of the water to the corresponding values shown on the abscissa. (See Table II.)

Content of Water and Chloride in Various Tissues: Exchanges of both Water and Chloride Ions between Tissue Fluid and Environment

Since some question arose as to whether a considerable part of the observed changes in tissue Cl might be assignable to the mere presence

TABLE III

Water and chloride contents of gills and bodies (minus gills) of normal mussels.

Gills:	Wet weight	11.02 grams
	Dry weight	1.09 "
	H_2O	90.1%
	Cl (wet wt.)	1.32%
	Mols. Cl per liter tissue water	0.413
Bodies minus Gills:	Wet weight	59.25
	Dry weight	7.30
	H_2O	87.7%
	Cl (wet weight)	1.075%
	Mols. Cl per liter tissue water	0.345
Combined Total:	Wet weight	70.27
	Dry weight	8.39
	H_2O	88.06%
	Cl	1.11%
	Mols. Cl per liter tissue water	0.355

of the medium itself in the capillary tubes of the gill structures, analyses were made of water and chloride in (1) the gills and (2) the rest of the tissues en masse, of six mussels taken directly from stock tanks of running sea water. In consistency with the experiment, tissues could not in this case be rinsed in alcohol prior to analysis (hence the slightly elevated Cl values). Table III shows the results of these analyses, and brings out the fact that the gills, as dissected for analysis, possess only a slightly higher moisture content than do the other tissues; furthermore while the chloride content of the gills was about 23 per cent higher than that of the other tissues, their relative proportion of the total wet body weight was only about 15.6 per cent, so that they were responsible

Table IV

Water and chloride contents of foot and body tissues (minus foot) of mussels immersed for 25 hours in sea water of three concentrations. N series: normal sea water, Cl = 1.86 per cent; D series: diluted sea water, Cl = 0.97 per cent; C series: concentrated sea water, Cl = 2.80 per cent.

No.	Sex	Wet wt. foot	Wet wt. other tissues	Combined wet wt.	Dry wt. foot	Dry wt. other tissues	Combined dry wt.	Percentage H_2O foot	Percentage H_2O other tissues	Percentage H_2O whole body	Percentage Cl foot	Percentage Cl body	Percentage Cl whole
N 1	? immat.	0.27	10.01	10.28	0.06	1.36	1.42	77.8	86.4	86.2	.765	1.06	1.05
N 2	♂ (?)	0.255	12.83	13.09	0.06	1.72	1.78	76.5	86.6	86.4	.71	1.11	1.10
N 3	♀	0.24	10.12	10.36	0.06	1.44	1.50	75.0	85.8	85.5	.57	1.04	1.03
N 4	♂	0.20	9.72	9.92	0.05	1.36	1.41	75.0	86.0	85.8	—	1.10	—
N 5	♂	0.18	7.94	8.12	0.04	1.02	1.06	77.8	87.15	86.95	.89	1.10	1.09
N 6	♀	0.12	10.80	10.92	0.03	1.35	1.38	75.0	87.5	87.4	.66	1.10	1.10
				av.wt. = 10.45				av. = 76.1	av. = 86.6	av. = 86.4	av. = .72	av. = 1.09	av. = 1.08
D 1	♀	0.23	13.07	13.30	0.04	1.275	1.315	82.6	90.25	90.1	.32	0.51	.505
D 2	♀	0.43	15.03	15.46	0.07	1.51	1.58	83.7	89.95	89.8	.38	.48	.48
D 3	♂	0.365	13.10	13.465	0.065	1.64	1.705	82.2	87.5	87.3	.345	.50	.50
D 4	♂	0.50	15.79	16.29	0.08	1.60	1.68	84.0	89.9	89.7	.36	.51	.505
D 5	♂	0.43	14.45	14.88	0.07	1.65	1.72	83.7	88.6	88.4	.39	.465	.46
D 6	? immat.	0.255	10.78	11.035	0.04	1.11	1.15	84.3	89.7	89.6	.32	.50	.50
				av.wt. = 13.07				av. = 83.4	av. = 89.3	av. = 89.15	av. = .35	av. = .49	av. = .49
C 1	? immat.	0.19	10.96	11.15	0.05	1.58	1.63	73.7	85.6	85.4	1.15	1.60	1.59
C 2	♂	0.105	8.96	9.065	0.025	1.34	1.365	76.2	85.05	84.95	1.715	1.60	1.60
C 3	♀	0.365	8.31	8.675	0.10	1.41	1.51	72.6	83.1	82.6	0.96	1.50	1.48
C 4	♂	0.30	9.27	9.57	0.07	1.37	1.44	76.7	85.2	84.95	1.46	1.62	1.61
C 5	♂	0.405	11.77	12.175	0.10	1.63	1.73	75.3	86.15	85.8	1.15	1.42	1.41
C 6	♀	—	—	12.54	—	—	1.90	—	—	84.85		1.34	1.34
	(Foot atrophied to tiny nub)			av.wt. = 10.53				av. = 74.9	av. = 85.05	av. = 84.75	av. = 1.29	av. = 1.51	av. = 1.505

for bringing the total tissue chloride up from 1.075 per cent to 1.11 per cent, an increase of only about 3.5 per cent. Observed shifts in tissue chloride were therefore not due to environmental solution mechanically suspended in the gills.

The question of whether the change in tissue chloride concentration might be due, at least in part, to the osmotic interchange of major quantities of *water,* with or without the migration of Cl ions as well, was investigated in an experiment involving eighteen animals of the usual range of length (i.e. 92 to 110 mm.), the results of which appear in Table IV. Six mussels were immersed in ordinary sea water, six propped open in hypotonic water (" 50 per cent sea water "; 0.97 per cent Cl), and a third set of six propped open in hypertonic water wherein the Cl concentration was 2.80 per cent. After being kept in the respective, constantly aerated solutions for 25 hours, the animals were analyzed. The foot of each was severed with a sharp razor blade and analyzed separately from the other tissues, in order to determine whether

TABLE V

Weight of water and of chloride per unit weight of dry tissue; concentration of chloride per 100 grams of tissue water.

Series	Grams H_2O per gm. dry tissues			Grams Cl per gm. dry flesh			Grams Cl per 100 gm. tissue-water		
	foot	body	whole	foot	body	whole	foot	body	whole
N	3.18	6.46	6.35	0.030	0.081	0.079	0.94	1.25	1.24
D	5.025	8.34	8.22	0.021	0.046	0.045	0.42	0.55	0.55
C	2.98	5.69	5.55	0.051	0.101	0.099	1.72	1.77	1.78

such a relatively compact structure might show any considerable difference in chloride shift as compared with that of softer tissues. The dry weights of all tissues were obtained by keeping them overnight in tared containers placed in an electric oven at 105° C., then re-weighing; chloride was determined in the usual manner. In the table, the N series represent the normal control animals kept in sea water, the D series those placed in the diluted sea water, and the C series those immersed in the more concentrated solutions. Some significant trends are observed. The average water content of foot tissues and of whole bodies shows a consistent increase in the order which would have been expected from a consideration of the osmotic effects of the relative solutions, i.e., $D > N > C$; the relative chloride values of foot tissues or of whole bodies show the opposite order, i.e., $C > N > D$.

The exchanges of both water and chloride ions, under the conditions of the preceding experiment, are brought out in a quantitative way in Table V, showing for each of the three series, the ratios of average water content and average chloride content, in grams per gram of dry tissue.

Furthermore, by plotting the values for Cl concentration in grams per 100 grams of tissue water, against Cl concentrations in grams per 100 ml. of sea water solutions, we arrive at the nearly linear relationships shown in Fig. 5. The foot, which contains less water, less chloride, and lower concentration of the latter, normally constitutes only about 2 per cent of the total body weight, and exerts no significant influences upon the data collected from analyses of the whole body. Nevertheless,

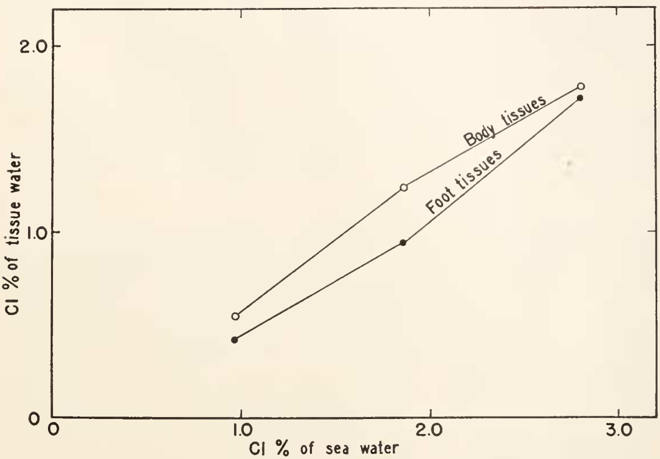

FIG. 5. Relationship between internal chloride concentrations in grams per cent of tissue water and in environmental water respectively. Sea water chlorinities lie within the range of values tolerated by mussels on sudden immersion.

this compact muscular tissue is observed to exchange both water and chloride with the aqueous environment in a manner similar to that of the other tissues.

Under the conditions of the above experiments, the mussel alters, throughout a physiologically wide range, the concentrations of its dissolved salts in such manner as to maintain a rather constant ratio between the Cl concentration of internal and that of external water, this ratio appearing to have an average value of the order of 1 : 1.60. The migration of both chloride and water occurs between the tissues and the solution outside.

Discussion

The ready exchange of water and chloride between the mussels' flesh and the environment may not involve any such profound changes in tissue cells themselves as might seem to be the case. The tissues of some organisms contain major quantities of the total chloride in the interstitial fluid, the cells themselves possessing the ion in very small amounts, and not readily undergoing alterations in water content or chloride concentration. This seems to be especially true in certain muscular tissues of mammals (Eggleton, Eggleton and Hamilton, 1937; Bourdillon, 1937), but some investigators are of the opinion that in other tissues (notably in frogs) considerable portion of the chloride is intracellular (Amberson, Nash, Mulder and Binns, 1938).

Since the completion of the present work, some informative results have been published by Steinbach (1940 *a, b*), who studied the content and distribution of water and electrolytes in the excised retractor muscles of certain marine invertebrates, i.e. the holothurian, *Thione briareus* and a sipunculid worm, *Phascolosoma*.

Steinbach immersed his material for a few hours in solutions containing chloride in concentrations of from 52 milli-equivalents per cent (i.e. full-strength sea-water) to nearly zero, employing isosmotic sucrose, or alternatively $NaNO_3$ solution as the sea water diluent. No hypertonic solutions were used. He obtained linear relationships throughout the experimental range of concentrations employed, the fresh *Thyone* tissue being richer in Cl (20.4 meq. Cl per cent) than that of *Phascolosoma* (9.1 meq. Cl per cent when fresh, and 16 meq. Cl per cent when soaked in sea water), and the former yielding steeper slopes of linear change. The normal water-content was very similar in both species, i.e. 75.9 per cent in *Thyone* and 78 per cent in *Phascolosoma* muscle.

The present writer immersed whole mussels for 24 hours or longer in normal, dilute, and concentrated solutions of natural sea salts within the range successfully tolerated by the animals. Linear relationships were apparent between the range of about 1.74 and 79.1 meq. Cl per cent of environmental water, when dealing with whole bodies, while such data as were collected upon the subsequently amputated foot alone revealed a nearly linear function between environmental Cl concentrations of 27.7 and 79.1 meq. Cl per cent.

It is also of interest to note that the initial concentration of Cl in Steinbach's *Thyone* muscle is virtually identical with that of the muscular foot of *Mytilus,* viz.; 20.4 and 20.3 meq. Cl per cent respectively, and that the water-contents of both are close, viz.: 75.9 per cent and 76.1 per cent. Furthermore, the Cl concentration in half-strength sea water

(Steinbach's fortified with isosmotic solutions of sucrose or $NaNO_3$, mine merely diluted) resulted in a decrease in *Thyone* muscle Cl to a value close to that determined for *Mytilus*-foot, viz.; 12 vs. 9.9 meq. Cl per cent respectively.

Steinbach's experiments led him to conclude that muscular tissues of both *Thyone* and *Phascolosoma* (classed as smooth muscle) contain sodium chloride almost exclusively in the extracellular space, that it is free to equilibrate by simple diffusion with external solutions, and that little if any can penetrate the tissue-cells themselves. He discusses the evidence in support of the conclusion that virtually all chloride is extracellular in the material studied by him, and in striated muscles of frogs and some other vertebrates as well, and that measurement of chloride content of such tissues may be employed as a relative measure of the extracellular space.

The muscular foot of *Mytilus* is observed to show osmotic behavior closely similar to that of the retractor muscles of *Thyone* and *Phascolosoma*, and must be very like the latter tissues in biochemical constitution and function.

The whole body of *Mytilus* shows a similar linear slope as does the foot tissue within the range of environmental Cl concentrations compared, but exhibits consistently higher water and Cl content. Deviations from the linear relationship between whole tissue Cl and sea water Cl which occur at extreme dilutions and extreme concentrations represent the failure of the physiological mechanism to control any longer the integrity of the chloride-free cellular space under relatively drastic conditions.

Investigations of biochemical or physiological changes in tissues of whole organisms, living successfully in controlled foreign environments, allow, in the conclusions drawn, a degree of certainty less frequently assured from data collected on isolated, surviving tissues. In the present instance, it is of much interest to note some close parallelisms between different species investigated by the respective experimental approaches.

The work reported in this paper was begun with the experimental ecological viewpoint in mind. In summarizing briefly, it is recalled that while the adult California mussel is able to adjust itself, with accompanying changes in tissue constitution, to a considerable range of salinities in the laboratory, this species, unlike *M. edulis,* is rarely if ever found in bays and estuaries, even though such waters may be considerably less diluted than were solutions which the animals have been shown experimentally to tolerate for indefinite periods.

Our findings during the course of this work suggested some experiments on the effect of dilute solutions of sea salts upon ripe sperm and eggs of this species, the process of fertilization, and subsequent development. Some preliminary experiments of this kind were accordingly carried out at the Scripps Institution by Dr. Robert T. Young, whose findings indicate that sea water diluted by more than 25 per cent may exert injurious effects upon (1) the sex products themselves, (2) incidence of fertilization, and (3) subsequent development. Doubtless the dilution of water in bays and estuaries is not the only factor responsible for the failure of *Mytilus californianus* to colonize them, but the sensitivity of eggs, sperm, and larvae to the dilute environments provides one tangible clue which should prove helpful in further attack on this and kindred problems.

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Summary

1. Experiments indicate that the adult mussel *Mytilus californiaus* is heterosmotic, yet potentially euryhaline to a considerable degree, although sperm, eggs and larvae are highly stenohaline toward dilution of the environment.

2. The tissue-chloride content is close to 1 per cent by wet weight, varying only slightly with season. Mature males show slightly higher chloride values than do the mature females, due to the higher chloride content of testicular tissues than of ovarian tissues.

3. Mussels can survive for indefinite periods the sudden and continued exposure of their tissues to sea water diluted by 50 per cent (Cl conc. 0.94 per cent) or water concentrated to half again its normal value (Cl conc. 2.73 per cent to 2.8 per cent). Below or above these two respective extremes, sudden immersion is fatal.

4. Within the limits of the physiologically tolerated range indicated, the concentrations of tissue chloride are adjusted to concentrations of chloride in the environment, with maintenance of an approximate value of 1 : 1.60, calculated as grams per 100 ml. of internal and external water.

5. Considerable individual differences exist in the rate of establishment of equilibrium between environmental and tissue chloride concentrations, when mussels are exposed to the indicated dilute and concentrated solutions.

6. While sudden immersion in solutions of sea salts below or above the respective limits resulted fatally, it was possible for mussels to survive in solutions considerably beyond such limits, i.e. in water diluted to as low as 0.62 per cent Cl, or concentrated to 3.48 per cent Cl, if the concentrations were altered by *gradual steps.*

7. Mussels surviving in sea water, gradually diluted to a chlorinity of 0.625 per cent Cl, underwent a fall in their tissue chloride to values of about 0.26 per cent to 0.38 per cent; animals kept in sea water gradually concentrated to a chlorinity of 3.48 per cent underwent a rise in their tissue chloride to average values of 2.25 per cent; at these respective points animals were at their threshold of tolerance and showed incipient sluggishness. Animals of such extreme chloride levels, however, recovered if placed in running sea water, and readily underwent therein a restoration of their tissue chlorides to normal values.

8. Exposure of mussels to the diluted or concentrated solutions results in migrations of both water and chloride between internal and external media.

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